

# Spectral properties of myoelectric signals from different motor units in the leg extensor muscles

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## Summary

Myoelectric signals measured using intramuscular electromyograms (EMGs) in animals have shown that faster motor units generate higher frequencies in their power spectra. However, evidence to relate myoelectric frequency and motor unit type from the surface electromyograms typically measured from man have remained elusive. The purpose of this study was to determine if spectral properties from surface EMG could be related to the different motor units in the muscles of the leg extensors in man. Reflex experiments (both tendon tap and electrically stimulated) and graded isometric contractions were used to generate muscle contractions with different patterns of motor unit recruitment. EMG was recorded from the vastus lateralis and medialis, rectus femoris, medial and lateral gastrocnemius and soleus muscles. The EMGs were resolved into their intensities in time–frequency space using wavelet techniques. The intensity spectra were calculated for the reflex responses

and for different contractile forces. The spectra were compared using principle component analyses and ANCOVA. Electrical stimulation can result in preferentially faster motor units being recruited, and in this study resulted in higher myoelectric frequencies than for the stretch reflex. During ramped contractions the motor units are recruited in an orderly fashion from slow to fast. As the faster motor units were recruited then higher frequency components appeared within the myoelectric intensity spectra. For all muscles tested there were significant correlations between the stage in contraction and the EMG frequency. Both approaches demonstrated higher frequency components in the myoelectric spectra when the faster motor units could be assumed to be active.

Key words: muscle, motor unit, wavelet, size-principle, principle component, PCA, ramped contraction, human.

## Introduction

Vertebrate skeletal muscle contractions are stimulated by motor unit action potentials (MUAPs), which travel along the sarcolemmas of the muscle fibres. The interference patterns that are created by the sum of the MUAPs form the basis of the myoelectric signal, which is detected by electromyography. The shape of the action potentials is a function of the relative rates of membrane depolarization to hyperpolarization. These relative rates depend on the ion flows through the voltage-gated Na<sup>+</sup> and K<sup>+</sup> channels which, in turn, can differ between the muscle fibre types. Indeed, both the conductance and resistance of the sarcolemma differ between fast and slow fibres in fish (Stanfield, 1972), amphibia (Adrian and Peachey, 1965), reptiles (Proske and Vaughan, 1968) and mammals (Luff and Atwood, 1972). Therefore, it should be expected that different types of muscle fibre generate different MUAPs. The myoelectric signal contains information about the form of the MUAPs and thus can provide us with an insight about the electrophysiology of the active muscle.

Recent developments in myoelectric signal decomposition

into time–frequency space (e.g. Karlsson et al., 2000; von Tscharner, 2000) allow a level of detail to be resolved that has not previously been possible. During both cycling and running movements, bursts of muscle activity occur at distinct myoelectric frequencies within each gait cycle (von Tscharner, 2000; Wakeling et al., 2001; von Tscharner et al., 2003) and it has been suggested that these may represent the signals from different motor units (Wakeling et al., 2001). When faster motor units are active within a myoelectric signal then increases occur in both the mean or median myoelectric frequency (Wretling et al., 1987; Gerdle et al., 1988b; Kupa et al., 1995) and also the conduction velocity of the MUAPs (Sadoyama et al., 1988; Kupa et al., 1995). These results suggest that there is some intrinsic property of the muscle fibre which can be characterised within the myoelectric signal. We have recently shown in the rainbow trout, cat and rat that faster and slower motor units do, indeed, generate distinct high and low myoelectric frequency bands, respectively (Wakeling et al., 2002; Wakeling and Syme, 2002). However, these initial

experiments considered polarised situations within a muscle by comparing discrete populations of motor unit types. Mammalian skeletal muscle is commonly mixed with a range of different fibre types and hybrid fibre types (Schiaffino and Reggiani, 1994; Bottinelli and Reggiani, 2000) and it is likely that a range of different myoelectric frequencies are generated by the range of different fibre types.

There are different ways for testing the spectral properties of myoelectric signals from the different motor units in man. Motor units are typically recruited in a graded manner (Henneman et al., 1965) from the slowest to the fastest during ramped isometric contractions (Garnett et al., 1978; Andreassen and Arendt-Nielsen, 1987). Therefore, if the observation that higher and lower myoelectric frequencies are generated by faster and slower muscle fibre types holds true for man it should be expected that the EMG signals during a graded isometric contraction will contain sequentially higher frequency components as the faster motor units become recruited. Secondly, electrical stimulation can generate reversals in the recruitment order with the faster motor units being preferentially activated (Kanda et al., 1977; Stephens et al., 1978), and such contractions can be compared with low magnitude stretch reflexes where the slowest motor units will be active (Henneman et al., 1965). The purpose of this study was to use these two approaches to elicit varying motor unit recruitment and to quantify the spectral properties of the myoelectric signals from these muscle contractions when it could be assumed that different motor recruitment occurred. It was expected that the changes in myoelectric spectra would be subtle, and so principle component analysis was chosen as one method for quantifying the signals, because it is a powerful technique that can identify systematic changes in spectral properties (Ramsay and Silverman, 1997). The principle component approach was compared to a second technique where the frequency bands were identified that could distinguish activity from the different motor units during ramped contractions.

## Materials and methods

### *Subjects*

Ten physically active subjects (five males and five females; age  $29.6 \pm 1.6$  years; mean  $\pm$  S.E.M.) were tested. Subjects were students at the University of Calgary and gave their informed, written consent to participate in accordance with the University of Calgary's Conjoint Health Research Ethics Board policy on research using human subjects.

### *Electromyography*

Myoelectric activity was measured from the quadriceps muscles during isometric knee extension contractions, and on a separate day from the triceps surae during isometric plantarflexion contractions. For the quadriceps contractions the subjects sat on a chair with their thigh horizontal, their right knee flexed at  $75^\circ$  from full extension, and their ankle strapped to a dynamometer arm, which had its rotation axis aligned with

the flexion/extension axis of the knee. For the triceps surae contractions the subjects sat with their foot strapped to the dynamometer arm, which had its rotation axis aligned with the plantar/dorsiflexion axis of the ankle, the foot was held vertical, the shank horizontal and the knee flexion angle was  $23^\circ$ . An external force transducer (Omega Engineering, Inc., Stamford, CT, USA) was attached to the dynamometer arm to measure the torque generated during knee extension exercises. An oscilloscope monitored the force, and was initially set with a cursor indicating the maximum voluntary contraction for each subject. The force was continuously recorded at 3600 Hz on the data collection computer. The subjects performed isometric contractions, and were asked to gradually increase the force from zero to maximum (using visual feedback) over a 4 s period. Each subject performed three such contractions for the knee extensions and five such contractions for the ankle plantarflexions with a 2 min rest period between each. This protocol resulted in no significant difference in the peak force between the contractions. Contractile force was expressed as % maximum voluntary contraction (MVC).

On a separate occasion the myoelectric signals were recorded in the soleus muscles from the male subjects during reflex contractions. The subjects lay in a supine position with the ankle flexed at  $90^\circ$ . Ten stretch reflexes were elicited by striking the Achilles tendon with a plexor. A further ten Hoffman (H-) reflexes were elicited by using electrical stimulation to the skin overlying the tibial nerve at the crease behind the knee *via* a bipolar electrode. Each stimulus consisted of a single 0.5 ms pulse (S88 stimulator and SIU 8T stimulus isolation unit, Grass-Telefactor, West Warwick, RI, USA), with the voltage set to the minimum required to result in a twitch of the foot.

Myoelectric activity was measured from the muscle bellies of the vastus medialis, rectus femoris, vastus lateralis, lateral gastrocnemius, medial gastrocnemius and soleus using round bipolar surface electrodes (Ag/AgCl) after prior removal of the hair and cleaning of the skin with isopropyl wipes. Each electrode was 10 mm in diameter and had an interelectrode spacing of 22 mm. Electrodes were placed midway between the motor end point (as determined in pilot experiments) and the distal end of the muscle belly. A ground electrode was placed on the fibular head. The EMGs were preamplified at source (bandwidth 10–500 Hz, 3 dB; Biovision, Wehrheim, Germany). Myoelectric signals were recorded at 3600 Hz, on a DAQCard-6062E 12-bit data acquisition card (National Instruments Corp., Austin, TX, USA). Both the EMG amplifiers and the recording computer were powered from batteries in order to minimize 60 Hz noise from external power sources.

### *Signal analysis*

The correlation coefficient,  $r$ , was estimated from the peak value of the cross-correlation function of the raw myoelectric signals between the muscle pairs for each ramped trial. The myoelectric signals were resolved into their myoelectric intensities in time–frequency space using wavelet techniques (von Tscharner, 2000). A set of 13 wavelets was used with

center frequencies,  $f_c$ , ranging from 7 Hz (wavelet 0) to 542 Hz (wavelet 12). The intensity is a measure of the time-varying power of the signal contained within a given frequency band. The intensity spectrum is a close approximation of a power spectrum calculated using traditional Fourier analysis. A wavelet domain was defined as the time series of intensity resolved for one wavelet only. The intensity spectrum for each reflex test was normalized to unit area.

For the ramped contractions the maximum myoelectric intensity that occurred across all frequencies was identified during each contraction. A threshold was set at 1% of this maximum and the time–frequency coordinates were identified for each peak that exceeded this threshold. The force at which each peak intensity occurred was then calculated. The force–frequency characteristics were plotted for each peak that occurred in the intensity. For each trial the intensity spectra for each sample point were pooled into bins according to the force level: 5–15, 15–25, 25–35, 35–45, 45–55, 55–65, 65–75, 75–85 and 85–95% MVC. The mean spectra were then calculated for each force bin, normalized so the spectrum from the 85–95% bin had unit area, and compiled into a matrix of data **A**.

#### Principle component analysis of the intensity spectra

The intensity spectra were compiled into a  $p \times N$  data matrix **A**. For this analysis there were  $N=2160$  spectra from the different force bins, trials, subjects and muscles for the ramped contractions and there were  $N=100$  spectra from the different stimuli and subjects for the reflex experiments. Each spectrum contained  $p=13$  intensities corresponding to the number of wavelets. The principle components of the data were determined (Morrison, 1967) from the covariance matrix **B** of the data **A**. Briefly, the principle component weightings of the data **A** are given by the unit eigenvectors  $\xi$  of the covariance matrix **B**. The importance of each component is given by the eigenvalue for each eigenvalue–eigenvector pair, with the greatest absolute eigenvalues corresponding to the most principle components.

In principle component analysis the mean is typically

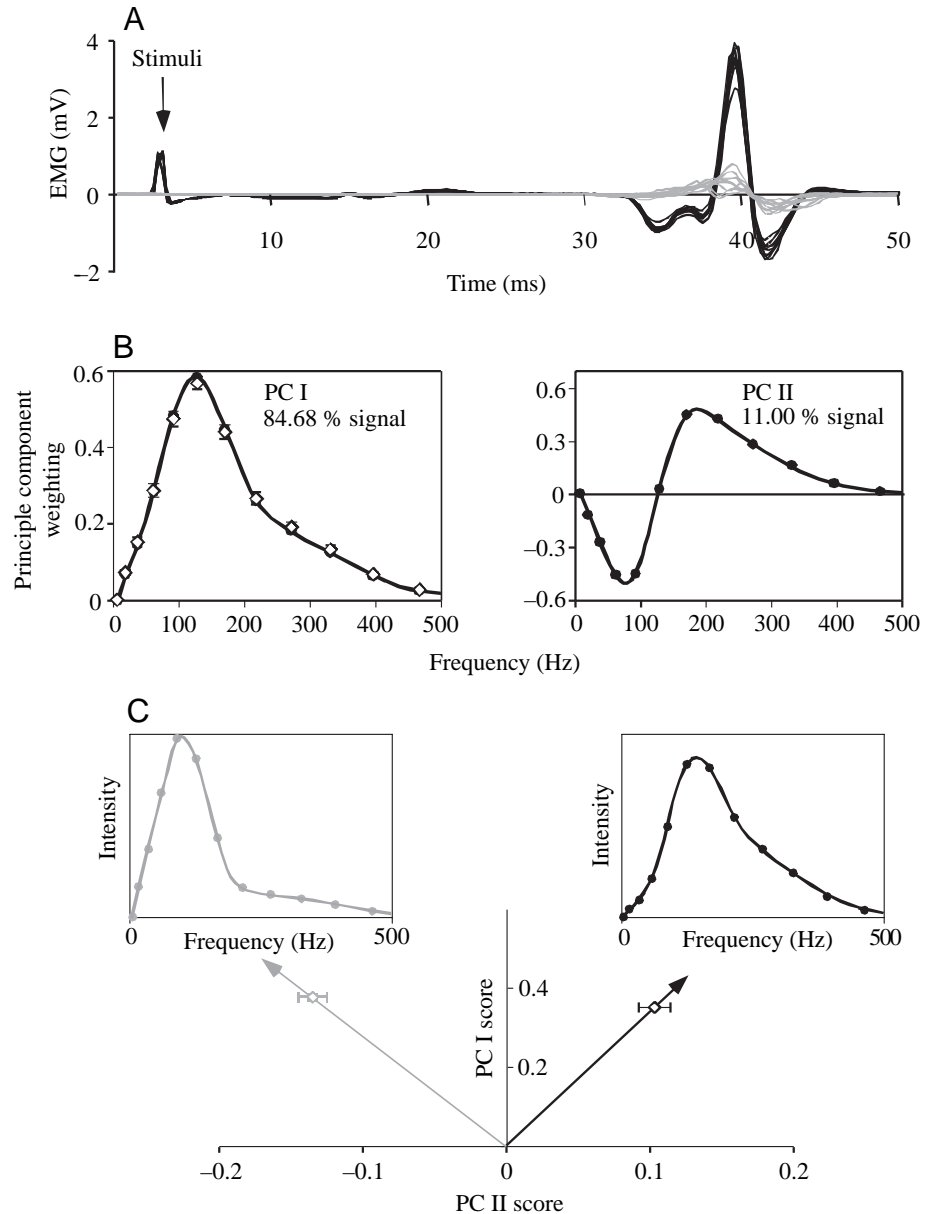


Fig. 1. Myoelectric signals and principle components from the electrical stimulation and tendon tap recordings from the soleus. (A) Myoelectric signals for the electrical stimulation (black lines) and tendon taps (gray lines) for the ten trials for one subject. The artefact for the electrical stimulation is shown by the arrow. (B) The weightings for the first two principle components (PC) are shown that describe the intensity spectra for both the electrical stimulation and tendon tap trials for all subjects ( $N=100$ ), with the relative proportion of the total signal that they describe. The principle component weightings are shown by the solid circles and line. The mean  $\pm$  S.E.M. intensity spectra for the data ( $N=100$ ) are shown by the open diamonds. (C) Mean  $\pm$  S.E.M. scores for PC I and PC II for the electrical stimulation (black symbols) and tendon taps (gray symbols) for all subjects ( $N=50$ ). The arrows mark the vectors in the PC I–PC II scoring plane, which form the reconstructed spectra.

subtracted from the data in an initial step, and so the eigenvectors describe the set of orthogonal components that maximize the variance of the data from the mean (Ramsay and Silverman, 1997). In this analysis, however, the mean was not subtracted. Therefore, the eigenvalues describe the set of orthogonal components that maximize the variability of the

entire data. The most principle component describes the greatest proportion of the data. The relative proportion of the data explained by each component is given by  $\xi' B \xi$ , and the principle component scores for each component for a given trial are given by  $\xi' A$ .

### Statistics

Analysis of variance (ANOVA) was used to determine the effect of reflex type and subject on the PC II scores for the reflex experiments. Analysis of covariance (ANCOVA) was used to determine the effect of the muscle type, subject and contraction force (the covariate) for each of the principle component, PC, scores. ANCOVA was used to determine the effect of the muscle type, subject and PC I score (the covariate) for the PC II score. The force at which the first peak of intensity occurred at each frequency band (wavelet domain) was determined for each trial. ANCOVA was used to determine the effect of the muscle type, subject and myoelectric frequency (the covariate) for this contraction force. Third order polynomial least-squares regression analyses were performed on the mean forces at which the initial peaks occurred for each muscle as a function of their myoelectric frequency. The effect of subject gender on the force–frequency response of the vastus lateralis muscle was tested with a general linear model ANOVA in which subject gender, EMG frequency and a gender–frequency interaction term were used as factors and the contraction force at which the initial peaks occurred was used as the response variable. Tests were considered significant at the  $\alpha=0.05$  level. Mean values are presented as mean  $\pm$  standard error of sample mean (S.E.M.).

### Results

The first two principle components for the reflex experiments in the soleus explained over 95% of the myoelectric signal. The first principle component had positive weightings for all frequencies with its peak intensity at wavelet domain 5 ( $f_c=128$  Hz; Fig. 1B). The shape of this component was similar to the mean intensity spectrum for the data

Table 1. Maximum correlation coefficients,  $r$ , from the cross-correlation functions between the raw myoelectric signals from adjacent muscles

	Medial gastrocnemius	Lateral gastrocnemius
Soleus	0.02 $\pm$ 0.01	0.01 $\pm$ 0.01
Lateral gastrocnemius	0.03 $\pm$ 0.01	
	Vastus medialis	Vastus lateralis
Rectus femoris	0.17 $\pm$ 0.05	0.02 $\pm$ 0.03
Vastus lateralis	0.03 $\pm$ 0.06	

Values are means  $\pm$  S.E.M. ( $N=30$  for quadriceps,  $N=50$  triceps surae).

(Fig. 1B). The second principle component contained negative weightings at wavelet domain 4 ( $f_c=92$  Hz) and below and positive weightings at wavelet domain 5 ( $f_c=128$  Hz) and above. The PC II scores were significantly greater for the H-reflexes than for the tendon-tap reflexes, and the combined spectra summed from the PC I and PC II scores demonstrated a higher frequency signal from the H-reflexes than for the tendon-tap reflexes (Fig. 1C).

The myoelectric activity showed a qualitative increase during each graded increase in contraction force (Fig. 2). Time–frequency analysis showed a graded increase in the high frequency components of myoelectric intensity with the increases in force (Fig. 2D). Careful observation of this intensity plot reveals the transient nature of the bursts of muscle activity. The mean correlation coefficients from the cross-correlation functions between the raw myoelectric signals of the muscles tested are given in Table 1, and were

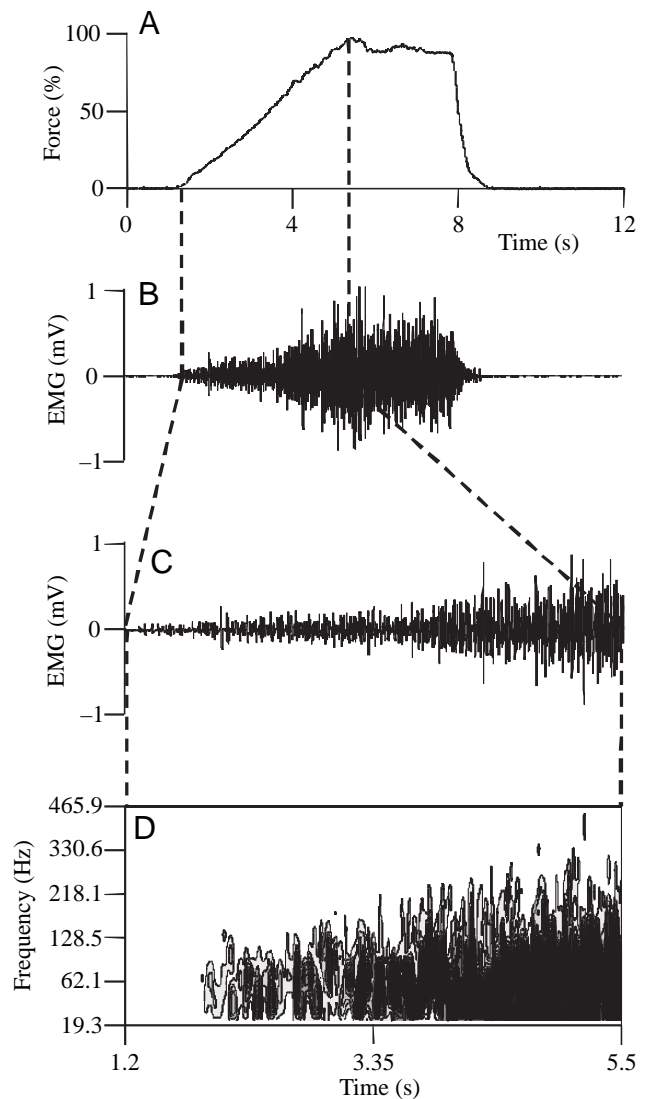


Fig. 2. Force (A) and EMG from vastus medialis (B,C) during a 4 s ramped isometric contraction. Increasing EMG intensity is shown by darker regions in time–frequency space (D).



less than 0.17 for the quadriceps and less than 0.03 for the triceps surae.

The first four principle components accounted for over 95% of the signal (Fig. 3). The first principle component was positive for all frequencies, with its peak weighting between 60–90 Hz. The second principle component contained negative weightings at wavelet domain 3 ( $f_c=62$  Hz) and below and positive weightings at wavelet domain 4 ( $f_c=92$  Hz) and above. The mean principle component scores across all force bins for these principle components are shown in Fig. 4. The PC I scores were positive for all muscles, with higher values for the triceps surae than for the quadriceps. There were small mean scores (although with different signs) for PC II across all muscles, and negligible scores for PC III and for PC IV for some muscles.

When the mean scores for PC I and PC II for the ramped contractions were plotted then a difference could be detected between the proximal and distal muscle groups (Fig. 5). The quadriceps muscles all showed similar ratios between PC I and PC II scores, with increases in the scores for the higher levels of contraction. On the other hand, the muscles from the triceps surae showed much less correlation between PC I and PC II scores with the level of contraction. When tested across all muscles, ANCOVA (Table 2) showed that the force level contributed significantly to the PC I scores with a positive correlation between force and PC I score. The force level did not contribute significantly to the score for principle components II, III and IV. Analysis of covariance showed no significant covariance between the PC I score and the PC II score.

Fig. 6 shows that as the contraction progressed, the initial peaks in intensity at each frequency band first occurred at lower frequencies and then progressively at higher frequencies (for  $k \leq 3$ ) as the contraction force increased. ANCOVA showed that there was a significant effect of the EMG frequency on the force of these initial peaks, with increases in force correlating to increases in the myoelectric frequency ( $P < 0.001$ ), and there was a significant difference in the response between the muscles ( $P < 0.001$ ). The results for the third order regression analysis of the mean responses for the six muscles tested (Fig. 7, Table 3) showed that the increases in the force–frequency relationship occurred (section between the minima and maxima) on average between the frequencies of 80 Hz and 500 Hz.

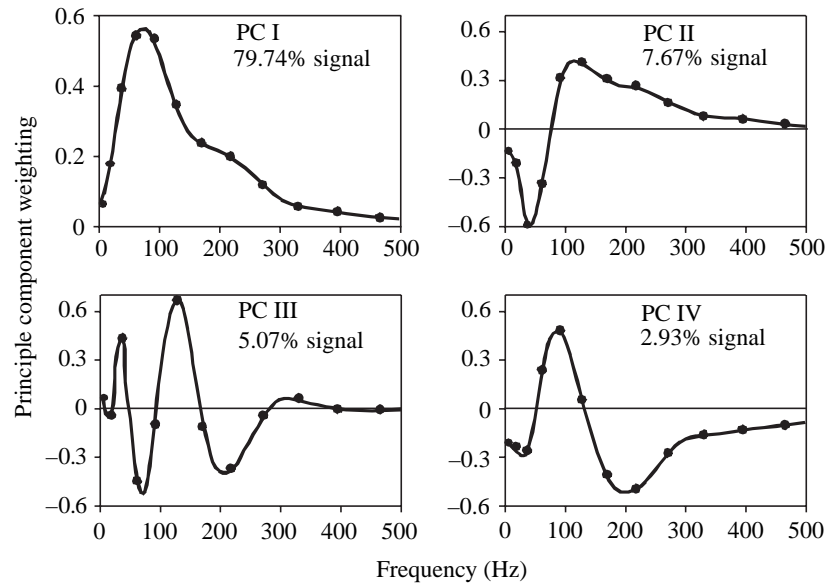


Fig. 3. Principle component weightings from the intensity spectra from the ramped isometric contractions. The components were calculated from 2160 intensity spectra from the rectus femoris, vastus lateralis, vastus medialis, lateral gastrocnemius, medial gastrocnemius and soleus muscles from 10 subjects and over a force range from 5 to 95% max. voluntary contraction (MVC). The first four principle components (PC) are shown, with the relative proportion of the total signal that they describe.

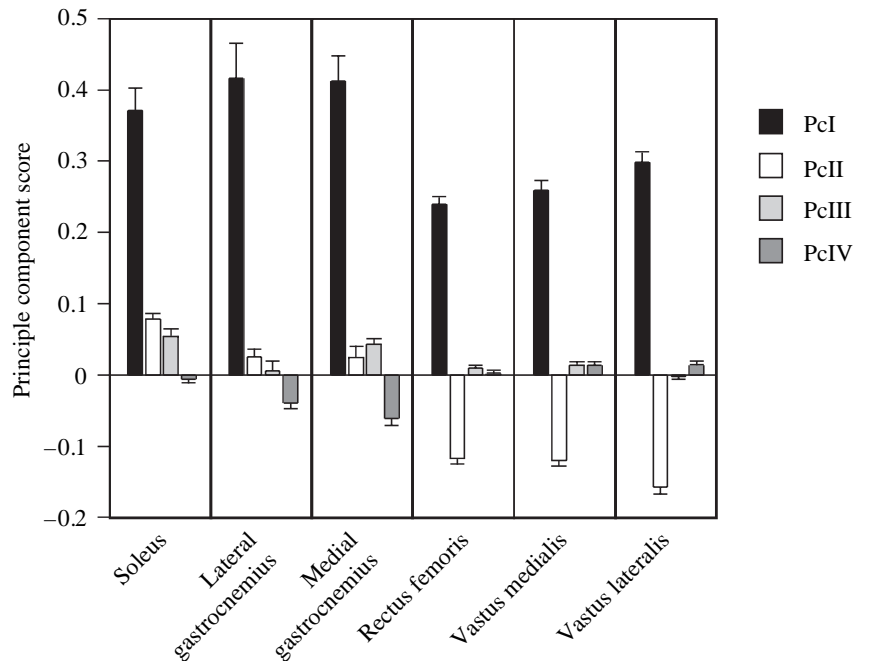


Fig. 4. Mean scores for the first four principle components. For each muscle the mean scores across all forces are shown for PC I in the left bar through to PC IV in the right bar. Bars show the mean  $\pm$  s.e.m. ( $270 \leq N \leq 450$ ).

There was no significant effect of subject gender on the force levels at which the initial peaks in myoelectric intensity occurred for the vastus lateralis muscle (ANOVA).

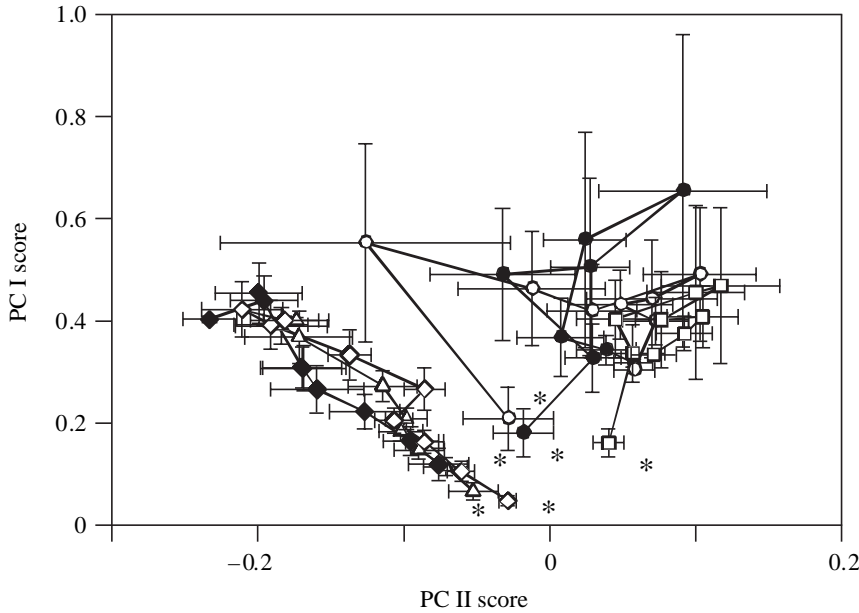


Fig. 5. Principle component scores from the intensity spectra from the ramped isometric contractions. The points denote the mean  $\pm$  S.E.M. scores ( $30 < N < 50$ ) for each force bin for the rectus femoris (open triangles), vastus lateralis (solid diamonds), vastus medialis (open diamonds), lateral gastrocnemius (solid circles), medial gastrocnemius (open circles) and soleus (open squares). The asterisks mark the 5–15% force bins, and the lines join sequentially higher force bins to the 85–95% bin.

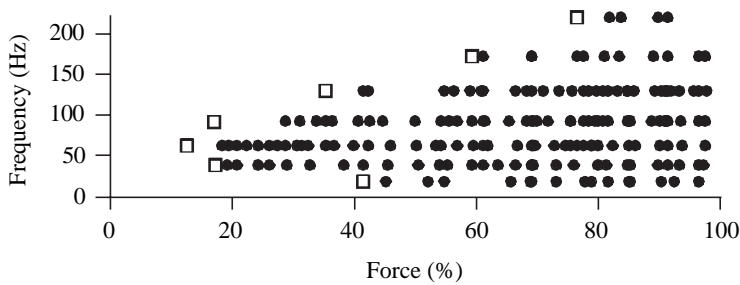


Fig. 6. Intensity peaks for the contraction in Fig. 2. Symbols show the peaks in EMG intensity at the frequency and contraction force at which they occurred. The initial peaks in each frequency band are shown by open squares; these peaks were used for further analysis.

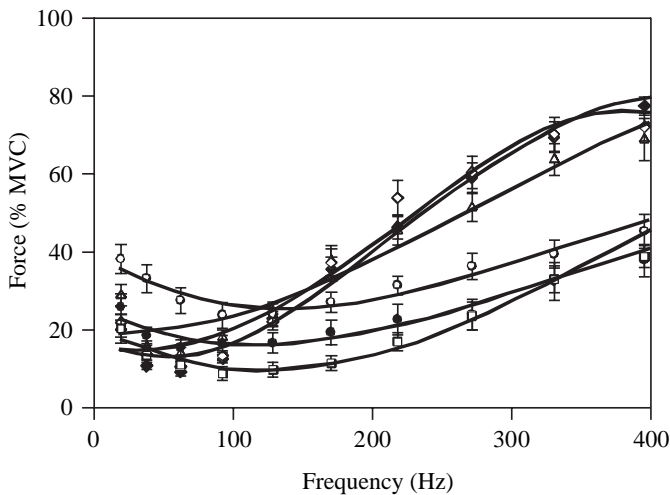


Fig. 7. Contraction force (% max. voluntary contraction; MVC) at which the initial intensity peak occurred during each graded contraction as a function of the center frequency for each wavelet domain. Rectus femoris, open triangles; vastus lateralis, solid diamonds; vastus medialis, open diamonds; lateral gastrocnemius, solid circles; medial gastrocnemius, open circles; soleus, open squares. Values are the mean  $\pm$  S.E.M. ( $30 \leq N \leq 50$  for  $f_c < 250$  Hz and  $6 \leq N \leq 50$  for  $250 \leq f_c \leq 400$  Hz). The third order polynomial regression lines (Table 3;  $P < 0.001$ ) were calculated from the mean points for each muscle and for  $1 \leq k \leq 13$  ( $19 \leq f_c \leq 542$  Hz).

**Discussion**

*Principle components of the myoelectric intensity spectra*

Spectral properties of myoelectric signals have traditionally been described by their power spectra, and quantified by the mean or median frequency of the power spectrum. The power spectrum from surface EMG in man forms a skewed bell-shaped curve with a maximum between about 50 to 150 Hz, and with the majority of the signal power at frequencies below 350 Hz (e.g. De Luca, 1997). The shape (and skewness) of the power spectrum changes with muscle fatigue (Petrofsky, 1979; Brody et al., 1991), and when the myoelectric signals are generated from different types of motor unit (Moritani et al., 1985; Gerdle et al., 1988b; Elert et al., 1992).

This study has shown that the principle components PC I and PC II may be used to quantify features of the muscle activity for both graded isometric contractions and also reflex responses. The fundamental properties of myoelectric spectra are conserved across isometric and dynamic contractions, and so it is suggested that principle component analysis will be useful tool for a range of myoelectric studies. PC I had a similar shape to the mean myoelectric intensity spectrum (Fig. 1B) and its scores can be used as an index of the magnitude of the intensity of the muscle contraction. PC II had negative and positive weightings, which transitioned at approximately 100 Hz (Figs 1, 3). The relative scores between PC I and PC II describe the main frequency components of each spectrum, with relatively higher PC II scores resulting in higher frequencies (Fig. 1C). The angle of the PC I-PC II scoring vector in the PC I-PC II scoring plane (Fig. 1C) provides a good measure of the frequency components for these myoelectric intensity spectra.

ANCOVA showed significant increases in the PC I scores as the force level increased during the ramped contractions (Table 2), showing that as the muscle activation was

Table 2. Probabilities from the analysis of covariance (ANCOVA) for the principle component scores

Factor	PC I	PC II	PC III	PC IV
Subject	<0.001	<0.001	0.284	<0.001
Muscle	0.001	<0.001	<0.001	<0.001
Force	<0.001	0.705	0.267	0.372

$P < 0.05$  indicates a significant effect of the factor on the principle component scores. The ANCOVAs tested the effect of 10 subjects, 6 muscles and 9 forces on 2160 intensity spectra.

progressively increased then increases occurred in the fundamental spectral intensity. The mean PC I score was greater for the muscles from the triceps surae than for the muscles from the quadriceps (Fig. 4). These mean scores were based on spectra that had been normalized to the intensity at the maximum force. Therefore, the distribution of PC I scores in Fig. 4 indicate that the triceps surae muscles reached maximal activation levels at lower forces within each ramped contraction than the muscles from the quadriceps, resulting in a higher mean score when considered over the whole contraction. These differences in PC I scores between muscles are consistent with the different motor unit recruitment strategies that occur between muscles. The smaller, more distal, muscles recruit all their motor units at lower relative forces than the larger more proximal muscles and so must rely on increases in motor unit firing rate to generate the higher forces (Kukulka and Clamann, 1981; de Luca et al., 1982).

Action potentials of the faster motor units have higher conduction velocities (Kupa et al., 1995; Sadoyama et al., 1988; Wakeling and Syme, 2002) and would contribute higher frequency components within the myoelectric signal. The taps of the Achilles tendon elicit stretch reflexes mediated by the muscle spindles, which recruit predominantly the slower motor units (Henneman et al., 1965; Garnett et al., 1978; Andreassen and Arendt-Nielsen 1987). The electrically stimulated H-reflex is an analog of the stretch reflex, sharing common pathways of muscle innervation. However, electrical stimulation results in the faster motor units being preferentially recruited (Kanda et al., 1977; Stephens et al., 1978), and this phenomenon can even occur within the time scale of the reflex (Burke et al.,

1984). The two forms of reflex response generate a synchronized motor unit action potential, but it can be expected that these potentials will be a lower frequency, slower motor unit, MUAP for the stretch reflex and a higher frequency, faster motor unit, MUAP for the electrically stimulated reflex. The results (Fig. 1C) supported this prediction and demonstrated that the differences in myoelectric frequency that result from altered innervation patterns can be resolved using the principle component approach.

During graded muscle contractions the motor units are activated in a graded manner (Henneman et al., 1965) with the faster motor units being recruited at higher levels of contraction (Garnett et al., 1978; Andreassen and Arendt-Nielsen, 1987). It should be expected that the recruitment of faster motor units at the higher forces results in higher frequencies within the myoelectric signal. However, previous studies investigating the dependence of mean myoelectric frequencies on contractile force during graded isometric contractions showed mixed results, with some papers reporting correlations (Bilodeau et al., 1991; Gerdle et al., 1988a; Moritani and Muro, 1987; Karlsson and Gerdle, 2001; Farina et al., 2002) whilst others reported lack of correlations (Bilodeau, 1991; Gerdle et al., 1988b; Onishi et al., 2000; Farina et al., 2002a). One reason for these conflicting results is that when measuring myoelectric signals with surface electrodes, the volume conductor effects of the tissue layers surrounding the muscle play a role in shaping the signal. Indeed, it has been shown that when the volume conductor effects of the tissues are taken into consideration then an invariance in mean frequency can be accounted for even when the MUAP conduction velocity increases (Farina et al., 2002a). In this study there was no significant effect of the contractile force on the PC II scores for the pooled data set, although Fig. 5 indicates that such a relation may occur for the quadriceps muscles. Increases in myoelectric frequency will be reflected in an increase in the scores of PC II relative to PC I, leading to a smaller angle of the PC I–PC II scoring vector (as illustrated in Fig. 1C). There was no systematic effect of the contractile force on the angle of the PC I–PC II scoring vector for the ramped contractions in this study (Fig. 5) and this is consistent with the studies reporting lack of correlation between force and mean myoelectric frequency. However, it should be noted that differences in myoelectric frequency could be discriminated in

Table 3. Results from the least-squares third order polynomial fits to the force–frequency points shown in Fig. 7

Muscle	$r^2$	$P$	Minimum		Maximum	
			Frequency (Hz)	Force (% MVC)	Frequency (Hz)	Force (% MVC)
Rectus femoris	0.964	<0.001	12.2	18.9	555.4	86.9
Vastus lateralis	0.985	<0.001	53.9	12.9	378.9	76.3
Vastus medialis	0.963	<0.001	32.4	14.7	415.3	79.8
Lateral gastrocnemius	0.967	<0.001	121.4	16.0	552.0	51.2
Medial gastrocnemius	0.967	<0.001	139.2	25.5	549.4	58.4
Soleus	0.986	<0.001	122.0	9.7	715.1	88.5

The regression results are for  $1 \leq k \leq 13$  ( $19 \leq f_c \leq 542$  Hz). MVC, maximum voluntary contraction.

the reflex experiments (Fig. 1) despite potential volume conductor effects, and so the reasons for the lack of correlation between force and myoelectric frequency during ramped contractions probably include other factors.

PC III and PC IV have significant scores for some, but not all muscles (Fig. 4). These components, along with the less important components, shape variations in the fine details of the intensity spectra between the different muscles. The raw intensity spectra for any given trial and force bin is the vector product of the principle component scores and the principle component weightings.

#### *Transient frequency bursts during myoelectric activity*

We have previously reported that different muscle fibre types produce signals with distinct myoelectric frequency bands and action potential conduction velocities for three animal species: the rainbow trout, cat and rat (Wakeling et al., 2002; Wakeling and Syme, 2002). Mammalian skeletal muscle is commonly mixed with a range of different fibre types and hybrid fibre types (Schiaffino and Reggiani, 1994; Bottinelli and Reggiani, 2000), and so it was expected that there should be myoelectric signals with a graded frequency response during the graded muscle contractions in this experiment. If it is possible to correlate a myoelectric frequency to the generation of a particular contractile force during an isometric contraction then that myoelectric frequency will correspond to the activity of motor units with a particular excitability within their motor unit pool.

The myoelectric signal showed intensity at progressively higher frequencies during the ramped contractions (Fig. 2D) and the higher frequency components correspond to the activity from the faster motor units. Careful observation of this figure shows that the myoelectric intensity occurs as a sequence of transient bursts, which can occur at any given frequency. For any given frequency band the bursts of activity occur in an irregular fashion (Fig. 2D), indicating that the motor units, once activated, do not fire consistently through the contraction. It is possible that variability in the firing of each motor unit during ramped contractions contributes to the variability in the instantaneous myoelectric frequency and the lack of correlation between myoelectric frequency and contractile force. This would be an interesting avenue for further investigation. Nonetheless, it should be noted that the higher frequency components are only present at the higher contractile forces.

When the forces were quantified at which the peaks in myoelectric intensity first exceeded the threshold (Fig. 6), there were significant correlations between force and frequency for all muscles (Fig. 7). These correlations occurred as a positive relation between the contractile force and the appearance of higher frequency spectral components in the myoelectric frequency between the frequencies of about 80 Hz and 400 Hz (Table 3). The force–frequency relation showed distinct patterns between the muscles of the quadriceps and triceps surae. The quadriceps showed a gradual increase in the force–frequency relation to forces in excess of 70% MVC whereas the triceps surae showed a much shallower relation, which

reached 40% MVC by the 400 Hz frequency. These results are again consistent with the observations that the larger more proximal muscles can recruit progressively faster motor units up to higher force levels than the smaller more distal muscles (Kukulka and Clamann, 1981; de Luca et al., 1982).

The myoelectric signals from a motor unit get broadened, and result in lower frequencies, as the muscle fibres get deeper within the muscle belly, due to volume conductor properties of the tissues (Roeleveld et al., 1997). These effects have led Farina and coworkers (Farina et al., 2002a) to demonstrate that the mean myoelectric frequency can remain constant even when there is a graded increase in conduction velocity of the constituent MUAPs. However, in this experiment we can still expect that the progressively higher frequency components in the myoelectric signal represent the action of progressively faster motor units. If part way through the contraction a faster motor unit was recruited then it would generate higher frequency components in the myoelectric signal if its muscle fibres were located near the surface electrodes, otherwise it would result in no frequency increase if its muscle fibres were located deeper within the muscle belly. Thus, part way through the contraction the higher frequency elements can only appear if progressively faster motor units are recruited whose fibres lie near the electrodes. The results show progressively higher frequencies appearing in the signal (Fig. 7) which, therefore, correspond to the progressively faster motor units recruited during all contractions. The volume conductor model predicts that if there is an inhomogeneous distribution of different fibre types within the muscle then the fibre distribution influences the spectral properties of the signal. However, certainly for the vastus lateralis, it has been shown that the slow type I and fast type II muscle fibres are randomly distributed across a muscle cross-section for 30-year-old adults (Lexell, 1993), and so in this experiment the fibre distribution should not be expected to present confounding factors.

Surface myoelectric recordings have a low spatial selectivity compared with intramuscular recordings, and so myoelectric signals can be recorded in locations far from their source (Lindström and Magnusson, 1977). Therefore, the effect of the cross-talk of both propagating and non-propagating signals should be considered when making interpretations about the results from surface electromyographic studies. For propagating signals, the frequency content depends on the distance from the source, with the tissues separating the sources from the electrodes acting as low-pass filters with cut-off frequency decreasing with increasing distance from source (Lindström and Magnusson, 1977). Therefore the cross-talk component of the signal will introduce low and not high frequency components into the myoelectric intensity spectra and will not contribute to the high frequency components which appear at higher forces. The effect of cross-talk from propagating signals can be quantified by the correlation coefficients from the cross-correlation functions of the raw signals between the muscles (Winter et al., 1993). In this study these correlation coefficients were below 0.17 for the quadriceps and 0.03 for the triceps surae (Table 1), but as discussed above this source of cross-talk will not influence the



force–frequency results (Fig. 7). On the other hand, the non-propagating components of cross-talk may introduce higher frequency components into the myoelectric intensity spectra (Farina et al., 2002b). However, we can ignore the effect of non-propagating cross-talk in this study for two reasons. Firstly, the high frequency signal introduced by the cross-talk increases with the signal amplitude. However, in our ramped experiment we found no correlation between the scores of PC I, a measure of the myoelectric intensity, and PC II, a measure of the frequency content, and so there is no evidence to suggest that the higher frequencies concurred with the greatest myoelectric intensities. Secondly, Farina and coworkers (Farina et al., 2002b) quantified the magnitude of the non-propagating cross-talk across the quadriceps muscles when one muscle was electrically stimulated and observed that the greatest peak-to-peak cross-talk was 8% of the signal magnitude in the adjacent, stimulated muscle (for a single-differential recording with an inter electrode distance of 20 mm, comparable to the recording system in this study). An 8% peak-to-peak value for cross-talk is equivalent to 0.7% of the signal intensity, a function of the square of the signal (von Tschärner, 2000). In this study we chose a threshold value of 1% of the peak intensity to quantify the force–frequency peaks, and this threshold would therefore exclude the signal components that may be present from non-propagating cross-talk.

The results from this study show that as the contractile force increases then progressively higher frequency components appear within the myoelectric signal (Fig. 7). The increase in force is initially achieved by increasing in the number of motor units recruited in the muscle, with the motor units being recruited in order from the slowest to the fastest. Therefore, the higher frequency components within the myoelectric signal correspond to the activity from the faster motor units. These results support the previous suggestions that the distinct, transient, bursts of high and low frequency myoelectric intensity that can be observed during locomotion (peaking at about 50 and 170 Hz; Wakeling et al., 2001; von Tschärner et al., 2003) result from activity of different types of motor units (Wakeling et al., 2001).

#### *Generation of myoelectric spectral properties*

It is commonly proposed that the reason for the higher conduction velocity of the motor unit action potentials in faster muscle fibres is due to their larger diameters. This idea can be explained using a local circuit model, which assumes the fibres are surrounded by a large volume of conducting fluid (Hodgkin, 1954), and has been confirmed by *in vitro* measurements of isolated muscle fibres in a large volume of conducting fluid (Håkansson, 1956). However, when the external conductance was modeled as being confined to the interstitial spaces, as in the *in vivo* condition, it was predicted that there should be no relation between fibre diameter and conduction velocity (Buchthal et al., 1955). To our knowledge, there is no evidence to indicate that the MUAP conduction velocity is related to muscle fibre diameter *in vivo*. Indirect evidence can be added to this debate using the data in this study.

Previously published histological studies of the vastus

lateralis have shown that the type I fibres have smaller cross-sectional area than the type II fibres for male subjects, but are bigger than the type II fibres for female subjects (Gerdle et al., 1997). Therefore, if the increase in conduction velocity of the motor unit action potentials were due to increases in fibre diameter, or area, then it should be expected that the recruitment of progressively faster motor units during a graded contraction would result in a gradual increase in the mean frequency for the male and a decrease in mean frequency for the female subjects (Karlsson and Gerdle, 2001). However, it has previously been demonstrated that there was no difference in the direction of the relationship between mean frequency and muscle force during ramped isometric contractions (Karlsson and Gerdle, 2001). This was confirmed in this study in that there is no significant effect of subject gender on the relation between the contractile force and the specific frequency bands at which the newly recruited fibres are activated. A dissociation between muscle fibre diameter and the conduction velocity of the action potentials or mean EMG frequency has also been reported for the quadriceps in populations with different proportions of each fibre-type (Wretling et al., 1987; Sadoyama et al., 1988; Linsen et al., 1991). The results from this study, therefore, support previous observations that EMG frequency can be dissociated from the muscle fibre diameter. It has been suggested that variation in conduction velocities is more closely linked to the differences in muscle fibre membrane properties across different fibre types than differences in fibre diameter (Buchthal et al., 1955). This is supported by direct evidence showing no relation between fibre diameter and EMG frequency in the myotomal muscle of fish, even when there was a 2.7-fold increase in EMG frequency between the slow and fast fibres (Wakeling et al., 2002).

#### *Conclusions*

Myoelectric intensity spectra from isometric ramped and reflex contractions in the leg extensor muscles can be largely described by two principle components. PC I describes a fundamental spectral shape and the PC I score is a measure of the overall intensity of the muscle contraction. The relative scores of PC II describe the frequency content of the spectra. A greater relative PC II score occurs during electrically stimulated contractions, when it was assumed that the higher myoelectric frequencies correspond to the recruitment of faster motor units. During ramped isometric contractions there is a progressive recruitment of faster motor units. The PC I scores increased with contractile force but there was no general relation between the force and the PC II score. This result is consistent with some of the previous studies that used the mean frequency of the power spectrum as a measure of the myoelectric frequency. However, as faster motor units are recruited, higher frequency components appear in the intensity spectra. The bursts of myoelectric activity at these higher frequencies correlate with the higher contractile force, or in the more general sense, with the progressively faster types of motor unit, which can be assumed to be recruited. Thus, during both the reflex experiments and the ramped isometric contractions the higher frequency components in the myoelectric

spectra corresponded to instances when it could be assumed that faster motor units were active.

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